Western Blot-Gel electrophoresis
Reagents:
NuPage LDS sample Buffer (4x): Invitrogen cat#46-5030 NP0007
NuPAGE 10%Bis-Tris Gel 1.0mmx10 well: Invotrogen, #NP0301
NuPage MOPS SDS Running Buffer: Invitrogen cat#46-5025 NP0001
Nitrocellulose: Protran BA85 Schleicher&Schuell cat#10402525N

Procedure:

Add sample buffer and samples to each tube, and then heat at 70^0C for 10 minutes.

1. **Electrophoresis**
   Set up gel box when waiting for sample to be heated up.

2. **Blotting**
   Transfer buffer: 3.5L

Tricine	**14.3 g**
Bis Tris	**18.3 g**
Na_2EDTA	**1g**
Add Ultrapure Water to 3 L	
Methenol	**70 ml**
Ultrapure Water	**to 4 L**
pH 7.2	

   Keep at 4^0C.

   a. **Construct: Back (-pole): black plastic, sponge pad, filter paper, gel, nitrocellulose**

membrane, filter paper, sponge pad, and lucent plastic :(+pole) Front

   b. **Two transfers: First: 30minutes at 100 volts**
      Second: 15 minutes at 100 volts
   c. **Keep current under 20 mA**
   d. **Take membrane out, put it into TBS, and store at 4^0C**

3. **Gel**
   a. **Take out gel after second transfer, put into GELCODE Blue Stain (Comassie blue, PIERCE cat# 24590).**
   b. **Stain the gels for 1 hour with gentle shaking, and then wash 3 times with distilled water. Dry the gels.**